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(71) Applicant *(for all designated States except US)*: **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PERSSON, Egon** [SE/SE]; Lundavägen 60, S-232 52 Åkarp (SE). **HANSEN, Carsten, Borgund** [DK/DK]; Platanhaven 69, DK-2600 Glostrup (DK).

(74) Common Representative: **NOVO NORDISK A/S**; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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(54) Title: NOVEL FORMULATIONS

(57) Abstract: The present invention relates to compositions comprising coagulation factor FVIIa.

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NOVEL FORMULATIONS

FIELD OF THE INVENTION

The present invention relates to novel formulations comprising coagulation factor VIIa as well as a methods for the administration of coagulation factor VIIa.

BACKGROUND OF THE INVENTION

Many proteins involved in the clotting cascade, including, e.g., Factor VII, Factor VIII, Factor IX, Factor X, and Factor XIII, are proving to be useful therapeutic agents to treat a variety of pathological conditions. Generally, the blood components which participate in what has been referred to as the coagulation "cascade" are proenzymes or zymogens, enzymatically inactive proteins which are converted to proteolytic enzymes by the action of an activator, itself an activated clotting factor. Coagulation factors that have undergone such a conversion and generally referred to as "active factors," and are designated by the addition of a lower case "a" suffix (e.g., activated factor VII (FVIIa)).

FVIIa, in contrast to other, homologous serine proteases, possesses an active conformation that is energetically unfavourable. The consequence is a far from optimal enzymatic activity of free wild type human Factor VIIa, which is dramatically enhanced upon binding to the cognate, membrane-bound cofactor tissue factor (TF). In the natural environment, the zymogenicity of free wild type human Factor VIIa ensures timely triggering and appropriate location of FVIIa haemostatic activity upon vascular lesion and concomitant TF exposure.

The traditional formulation of FVII only contain wild type human FVIIa. There is a need in the art for improved formulation of FVII, where the wild type human FVIIa is supplemented with FVII polypeptides with increased or different activity compared to wild type human Factor VIIa.

SUMMARY OF THE INVENTION

The present invention relates in a broad aspect to pharmaceutical compositions comprising wild type human FVIIa.

In a first aspect the present invention relates to a composition comprising wild type human FVIIa and a Factor VII related polypeptide.

In a second aspect the present invention relates to a method for the treatment of bleeding episodes in a subject or for the enhancement of the normal haemostatic system, the method comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of:

a) composition comprising wild type human FVIIa and a Factor VII related polypeptide; or

b) a first composition comprising wild type human FVIIa and a second composition comprising a Factor VII related polypeptide.

In a third aspect the present invention relates to a process for preparing a composition comprising wild type human FVIIa and a Factor VII related polypeptide, wherein the process comprises the step of: mixing wild type human FVIIa with a Factor VII related polypeptide in an aqueous medium.

In a further aspect the present invention relates to a use of a composition comprising wild type human FVIIa and a Factor VII related polypeptide for the preparation of a medicament for the treatment of bleeding episodes in a subject or for the enhancement of the normal haemostatic system.

DETAILED DESCRIPTION OF THE INVENTION

Recent advances in our understanding of the mechanisms regulating the activity of FVIIa have pinpointed side chains that function as zymogenicity determinants in the free enzyme. Replacements of these amino acid residues have resulted in FVIIa molecules with improved intrinsic (TF-independent) catalytic efficiency. The relatively high intrinsic activity of some of these FVIIa variants suggests that the zymogen-like conformation of free factor VIIa is dictated by a limited number of key amino acid residues. One of these superactive FVIIa variants, containing the mutations at positions 158, 296 and 298, exhibits several properties resembling TF-bound rather than free FVIIa. Apart from increased intrinsic enzymatic activity and inhibitor susceptibility as compared with wild-type FVIIa, these FVIIa variants have a diminished requirement for calcium ions and a more deeply buried protease domain N-terminus (Ile153) indicating salt bridge formation of this residue with Asp343.

The present invention relates to the finding that it may be an advantage to combine wild-type human FVIIa and a Factor VII related polypeptide with different properties compared to wild-type human FVIIa, such as FVIIa variants with increased proteolytic activity as compared to wild-type human FVIIa in a pharmaceutical composition for the treatment of bleedings.

As already suggested wild-type human FVIIa and FVIIa variants with increased proteolytic activity as compared to wild-type human FVIIa have different properties. Whereas free wild-type human FVIIa has a zymogen-like conformation, FVIIa variants with increased proteolytic activity as compared to wild-type human FVIIa exhibits several properties resembling TF-bound FVIIa.

In order to obtain a hemostatic composition of desired potency, a mixture of wild-type human FVIIa and a variant thereof can be conceived. For instance, if a pharmaceutical composition with a specific FVIIa activity 5-fold that of wild-type human FVIIa is considered optimal, and a FVIIa variant with this specific activity is not available, this can be achieved by mixing equal amounts of wild-type human FVIIa (activity = 1) and a variant thereof with a relative activity of 9. This approach is more accurate than simply administering a 5-fold higher dosis of FVIIa or a 2-fold lower dosis of a FVIIa variant with a relative activity of 10.

Furthermore, other parameters, such as pharmacokinetics and/or the distribution between membrane-bound FVIIa, free FVIIa in solution and TF bound FVIIa, may be affected by a change in dosis. By using the same amount of total FVIIa polypeptide protein in the pharmaceutical composition, this distribution between membrane-bound FVIIa, free FVIIa in solution and TF bound FVIIa may be kept constant.

Thus the present invention provides the ability to produce a FVIIa-based hemostatic composition with tailor-made potency and efficacy.

In one embodiment of the invention, the molar ratio between the wild type human FVIIa and the Factor VII related polypeptide is from about 1:99 to about 99:1.

In a further embodiment of the invention, the Factor VII related polypeptide has a proteolytic activity higher than wild type human FVIIa.

In a further embodiment of the invention, the Factor VII related polypeptide is selected from the group consisting of: L305V-FVII, L305V/M306D/D309S-FVII, L305I-FVII, L305T-FVII, F374P-FVII, V158T/M298Q-FVII, V158D/E296V/M298Q-FVII, K337A-FVII, M298Q-FVII, V158D/M298Q-FVII, L305V/K337A-FVII, V158D/E296V/M298Q/L305V-FVII, V158D/E296V/M298Q/K337A-FVII, V158D/E296V/M298Q/L305V/K337A-FVII, K157A-FVII, E296V-FVII, E296V/M298Q-FVII, V158D/E296V-FVII, V158D/M298K-FVII, and S336G-FVII, L305V/K337A-FVII, L305V/V158D-FVII, L305V/E296V-FVII, L305V/M298Q-FVII, L305V/V158T-FVII, L305V/K337A/V158T-FVII, L305V/K337A/M298Q-FVII, L305V/K337A/E296V-FVII, L305V/K337A/V158D-FVII, L305V/V158D/M298Q-FVII, L305V/V158D/E296V-FVII, L305V/V158T/M298Q-FVII, L305V/V158T/E296V-FVII, L305V/E296V/M298Q-FVII, L305V/V158D/E296V/M298Q-FVII, L305V/V158T/E296V/M298Q-FVII, L305V/V158T/K337A/M298Q-FVII, L305V/V158T/E296V/K337A-FVII, L305V/V158D/K337A/M298Q-FVII, L305V/V158D/E296V/K337A–FVII, L305V/V158D/E296V/M298Q/K337A-FVII, L305V/V158T/E296V/M298Q/K337A-FVII, S314E/K316H-FVII, S314E/K316Q-FVII, S314E/L305V-FVII, S314E/K337A-FVII, S314E/V158D-FVII, S314E/E296V-FVII, S314E/M298Q-FVII, S314E/V158T-FVII, K316H/L305V-FVII, K316H/K337A-FVII, K316H/V158D-FVII, K316H/E296V-FVII,

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F374Y/L305V/E296V/M298Q/K337A/V158T/S314E-FVII,
F374Y/L305V/V158D/E296V/M298Q/K337A/S314E-FVII, S52A-Factor VII, S60A-Factor VII; R152E-Factor VII, S344A-Factor VII, Factor VIIa lacking the Gla domain; and P11Q/K33E-FVII, T106N-FVII, K143N/N145T-FVII, V253N-FVII, R290N/A292T-FVII, G291N-FVII, R315N/V317T-FVII, K143N/N145T/R315N/V317T-FVII; and FVII having substitutions, additions or deletions in the amino acid sequence from 233Thr to 240Asn, FVII having substitutions, additions or deletions in the amino acid sequence from 304Arg to 329Cys. In one embodiment of the invention, the Factor VII related polypeptide is the activated FVIIa form of the peptide.

In a further embodiment of the invention, the molar ratio between wild type human FVIIa and the Factor VII related polypeptide is from about 10:90 to about 90:10, such as from about 20:80 to about 80:20, such as from about 30:70 to about 70:30, such as from about 40:60 to about 60:40.

In a further embodiment of the invention, the molar ratio between wild type human FVIIa and the Factor VII related polypeptide is from about 1:99 to about 10:90, such as from about 10:90 to about 20:80, such as from about 20:80 to about 30:70, such as from about 30:70 to about 40:60.

In a further embodiment of the invention, the molar ratio between wild type human FVIIa and the Factor VII related polypeptide is from about 99:1 to about 90:10, such as from about 90:10 to about 80:20, such as from about 80:20 to about 70:30, such as from about 70:30 to about 60:40.

In a further embodiment of the invention, the ratio between the proteolytic activity of the Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 1.25 when tested in assay 1 as described herein.

In a further embodiment of the invention, the ratio between the proteolytic activity of the Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 2.0, such as at least about 4.0, when tested in assay 1 as described herein.

In a further embodiment of the invention, the ratio between the proteolytic activity of the Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 1.25 when tested in assay 2 as described herein.

In a further embodiment of the invention, the ratio between the proteolytic activity of the Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 2.0, such as at least about 4.0, when tested in assay 2 as described herein.

As used herein, "wild type human FVIIa" is a polypeptide having the amino acid sequence disclosed in U.S. Patent No. 4,784,950 (figure 1), also referred to as recombinant human FVIIa (rhFVIIa) in the present specification.

As used herein, "Factor VII related polypeptide" refers to Factor VII polypeptides different from wild type human FVIIa. This includes variants of Factor VII exhibiting substantially the same or improved biological activity relative to wild-type Factor VII, as well as Factor VII derivatives and Factor VII conjugates. The term "Factor VII" is intended to encompass Factor VII polypeptides in their uncleaved (zymogen) form, as well as those that have been proteolytically processed to yield their respective bioactive forms, which may be designated Factor VIIa. Typically, Factor VII is cleaved between residues 152 and 153 to yield Factor VIIa. Such variants of Factor VII may exhibit different properties relative to human Factor VII, including stability, phospholipid binding, altered specific activity, and the like.

The term "Factor VII derivative" as used herein, is intended to designate wild-type Factor VII, variants of Factor VII exhibiting substantially the same or improved biological activity relative to wild-type Factor VII and Factor VII-related polypeptides, in which one or more of the amino acids of the parent peptide have been chemically modified, e.g. by alkylation, PEGylation, acylation, ester formation or amide formation or the like. This includes but are not limited to PEGylated human Factor VIIa, cysteine-PEGylated human Factor VIIa and variants thereof.

The term "PEGylated human Factor VIIa" means human Factor VIIa, having a PEG molecule conjugated to a human Factor VIIa polypeptide. It is to be understood, that the PEG molecule may be attached to any part of the Factor VIIa polypeptide including any amino acid residue or carbohydrate moiety of the Factor VIIa polypeptide. The term "cysteine-PEGylated human Factor VIIa " means Factor VIIa having a PEG molecule conjugated to a sulfhydryl group of a cysteine introduced in human Factor VIIa.

The biological activity of Factor VIIa in blood clotting derives from its ability to (i) bind to tissue factor (TF) and (ii) catalyze the proteolytic cleavage of Factor IX or Factor X to produce activated Factor IX or X (Factor IXa or Xa, respectively). For purposes of the invention, Factor VIIa biological activity may be quantified by measuring the ability of a preparation to promote blood clotting using Factor VII-deficient plasma and thromboplastin, as described, e.g., in U.S. Patent No. 5,997,864. In this assay, biological activity is expressed as the reduction in clotting time relative to a control sample and is

converted to "Factor VII units" by comparison with a pooled human serum standard containing 1 unit/ml Factor VII activity. Alternatively, Factor VIIa biological activity may be quantified by (i) measuring the ability of Factor VIIa to produce of Factor Xa in a system comprising TF embedded in a lipid membrane and Factor X. (Persson et al., J. Biol. Chem. 272:19919-19924, 1997); (ii) measuring Factor X hydrolysis in an aqueous system; (iii) measuring its physical binding to TF using an instrument based on surface plasmon resonance (Persson, FEBS Letts. 413:359-363, 1997) and (iv) measuring hydrolysis of a synthetic substrate.

Factor VII variants having substantially the same or improved biological activity relative to wild-type Factor VIIa encompass those that exhibit at least about 25%, preferably at least about 50%, more preferably at least about 75% and most preferably at least about 90% of the specific activity of Factor VIIa that has been produced in the same cell type, when tested in one or more of a clotting assay, proteolysis assay, or TF binding assay as described above.

Variants of Factor VII, whether exhibiting substantially the same or better bioactivity than wild-type Factor VII, include, without limitation, polypeptides having an amino acid sequence that differs from the sequence of wild-type Factor VII by insertion, deletion, or substitution of one or more amino acids.

The terms "variant" or "variants", as used herein, is intended to designate Factor VII having the sequence of wild type human FVII, wherein one or more amino acids of the parent protein have been substituted by another amino acid and/or wherein one or more amino acids of the parent protein have been deleted and/or wherein one or more amino acids have been inserted in protein and/or wherein one or more amino acids have been added to the parent protein. Such addition can take place either at the N-terminal end or at the C-terminal end of the parent protein or both. The "variant" or "variants" within this definition still have FVII activity in its activated form. In one embodiment a variant is 70 % identical with the sequence of wild type human FVII. In one embodiment a variant is 80 % identical with the sequence of wild type human FVII. In another embodiment a variant is 90 % identical with the sequence of wild type human FVII. In a further embodiment a variant is 95 % identical with the sequence of wild type human FVII.

Non-limiting examples of Factor VII variants having substantially the same biological activity as wild-type Factor VII include S52A-FVIIa, S60A-FVIIa (Lino et al., Arch. Biochem. Biophys. 352: 182-192, 1998); FVIIa variants exhibiting increased proteolytic stability as disclosed in U.S. Patent No. 5,580,560; Factor VIIa that has been proteolytically cleaved between residues 290 and 291 or between residues 315 and 316 (Mollerup et al., Biotechnol. Bioeng. 48:501-505, 1995); oxidized forms of Factor VIIa

(Kornfelt et al., Arch. Biochem. Biophys. 363:43-54, 1999); FVII variants as disclosed in PCT/DK02/00189; and FVII variants exhibiting increased proteolytic stability as disclosed in WO 02/38162 (Scripps Research Institute); FVII variants having a modified Gla-domain and exhibiting an enhanced membrane binding as disclosed in WO 99/20767 (University of Minnesota); and FVII variants as disclosed in WO 01/58935 (Maxygen ApS) and WO 04/029091 (Maxygen ApS).

Non-limiting examples of FVII variants having increased biological activity compared to wild-type FVIIa include FVII variants as disclosed in WO 01/83725, WO 02/22776, WO 02/077218, PCT/DK02/00635, Danish patent application PA 2002 01423, Danish patent application PA 2001 01627; WO 02/38162 (Scripps Research Institute); and FVIIa variants with enhanced activity as disclosed in JP 2001061479 (Chemo-Sero-Therapeutic Res Inst.).

Examples of variants of factor VII or factor VII-related polypeptides include, without limitation, L305V-FVII, L305V/M306D/D309S-FVII, L305I-FVII, L305T-FVII, F374P-FVII, V158T/M298Q-FVII, V158D/E296V/M298Q-FVII, K337A-FVII, M298Q-FVII, V158D/M298Q-FVII, L305V/K337A-FVII, V158D/E296V/M298Q/L305V-FVII, V158D/E296V/M298Q/K337A-FVII, V158D/E296V/M298Q/L305V/K337A-FVII, K157A-FVII, E296V-FVII, E296V/M298Q-FVII, V158D/E296V-FVII, V158D/M298K-FVII, and S336G-FVII, L305V/K337A-FVII, L305V/V158D-FVII, L305V/E296V-FVII, L305V/M298Q-FVII, L305V/V158T-FVII, L305V/K337A/V158T-FVII, L305V/K337A/M298Q-FVII, L305V/K337A/E296V-FVII, L305V/K337A/V158D-FVII, L305V/V158D/M298Q-FVII, L305V/V158D/E296V-FVII, L305V/V158T/M298Q-FVII, L305V/V158T/E296V-FVII, L305V/E296V/M298Q-FVII, L305V/V158D/E296V/M298Q-FVII, L305V/V158T/E296V/M298Q-FVII, L305V/V158T/K337A/M298Q-FVII, L305V/V158T/E296V/K337A-FVII, L305V/V158D/K337A/M298Q-FVII, L305V/V158D/E296V/K337A–FVII, L305V/V158D/E296V/M298Q/K337A-FVII, L305V/V158T/E296V/M298Q/K337A-FVII, S314E/K316H-FVII, S314E/K316Q-FVII, S314E/L305V-FVII, S314E/K337A-FVII, S314E/V158D-FVII, S314E/E296V-FVII, S314E/M298Q-FVII, S314E/V158T-FVII, K316H/L305V-FVII, K316H/K337A-FVII, K316H/V158D-FVII, K316H/E296V-FVII, K316H/M298Q-FVII, K316H/V158T-FVII, K316Q/L305V-FVII, K316Q/K337A-FVII, K316Q/V158D-FVII, K316Q/E296V-FVII, K316Q/M298Q-FVII, K316Q/V158T-FVII, S314E/L305V/K337A-FVII, S314E/L305V/V158D-FVII, S314E/L305V/E296V-FVII, S314E/L305V/M298Q-FVII, S314E/L305V/V158T-FVII, S314E/L305V/K337A/V158T-FVII, S314E/L305V/K337A/M298Q-FVII, S314E/L305V/K337A/E296V-FVII, S314E/L305V/K337A/V158D-FVII, S314E/L305V/V158D/M298Q-FVII, S314E/L305V/V158D/E296V-FVII, S314E/L305V/V158T/M298Q-FVII,

S314E/L305V/V158T/E296V-FVII, S314E/L305V/E296V/M298Q-FVII,
S314E/L305V/V158D/E296V/M298Q-FVII, S314E/L305V/V158T/E296V/M298Q-FVII,
S314E/L305V/V158T/K337A/M298Q-FVII, S314E/L305V/V158T/E296V/K337A-FVII,
S314E/L305V/V158D/K337A/M298Q-FVII, S314E/L305V/V158D/E296V/K337A-FVII,
S314E/L305V/V158D/E296V/M298Q/K337A-FVII,
S314E/L305V/V158T/E296V/M298Q/K337A-FVII, K316H/L305V/K337A-FVII,
K316H/L305V/V158D-FVII, K316H/L305V/E296V-FVII, K316H/L305V/M298Q-FVII,
K316H/L305V/V158T-FVII, K316H/L305V/K337A/V158T-FVII,
K316H/L305V/K337A/M298Q-FVII, K316H/L305V/K337A/E296V-FVII,
K316H/L305V/K337A/V158D-FVII, K316H/L305V/V158D/M298Q-FVII,
K316H/L305V/V158D/E296V-FVII, K316H/L305V/V158T/M298Q-FVII,
K316H/L305V/V158T/E296V-FVII, K316H/L305V/E296V/M298Q-FVII,
K316H/L305V/V158D/E296V/M298Q-FVII, K316H/L305V/V158T/E296V/M298Q-FVII,
K316H/L305V/V158T/K337A/M298Q-FVII, K316H/L305V/V158T/E296V/K337A-FVII,
K316H/L305V/V158D/K337A/M298Q-FVII, K316H/L305V/V158D/E296V/K337A –FVII,
K316H/L305V/V158D/E296V/M298Q/K337A-FVII,
K316H/L305V/V158T/E296V/M298Q/K337A-FVII, K316Q/L305V/K337A-FVII,
K316Q/L305V/V158D-FVII, K316Q/L305V/E296V-FVII, K316Q/L305V/M298Q-FVII,
K316Q/L305V/V158T-FVII, K316Q/L305V/K337A/V158T-FVII,
K316Q/L305V/K337A/M298Q-FVII, K316Q/L305V/K337A/E296V-FVII,
K316Q/L305V/K337A/V158D-FVII, K316Q/L305V/V158D/M298Q-FVII,
K316Q/L305V/V158D/E296V-FVII, K316Q/L305V/V158T/M298Q-FVII,
K316Q/L305V/V158T/E296V-FVII, K316Q/L305V/E296V/M298Q-FVII,
K316Q/L305V/V158D/E296V/M298Q-FVII, K316Q/L305V/V158T/E296V/M298Q-FVII,
K316Q/L305V/V158T/K337A/M298Q-FVII, K316Q/L305V/V158T/E296V/K337A-FVII,
K316Q/L305V/V158D/K337A/M298Q-FVII, K316Q/L305V/V158D/E296V/K337A –FVII,
K316Q/L305V/V158D/E296V/M298Q/K337A-FVII,
K316Q/L305V/V158T/E296V/M298Q/K337A-FVII, F374Y/K337A-FVII, F374Y/V158D-FVII,
F374Y/E296V-FVII, F374Y/M298Q-FVII, F374Y/V158T-FVII, F374Y/S314E-FVII,
F374Y/L305V-FVII, F374Y/L305V/K337A-FVII, F374Y/L305V/V158D-FVII,
F374Y/L305V/E296V-FVII, F374Y/L305V/M298Q-FVII, F374Y/L305V/V158T-FVII,
F374Y/L305V/S314E-FVII, F374Y/K337A/S314E-FVII, F374Y/K337A/V158T-FVII,
F374Y/K337A/M298Q-FVII, F374Y/K337A/E296V-FVII, F374Y/K337A/V158D-FVII,
F374Y/V158D/S314E-FVII, F374Y/V158D/M298Q-FVII, F374Y/V158D/E296V-FVII,
F374Y/V158T/S314E-FVII, F374Y/V158T/M298Q-FVII, F374Y/V158T/E296V-FVII,
F374Y/E296V/S314E-FVII, F374Y/S314E/M298Q-FVII, F374Y/E296V/M298Q-FVII,
F374Y/L305V/K337A/V158D-FVII, F374Y/L305V/K337A/E296V-FVII,

F374Y/L305V/K337A/M298Q-FVII, F374Y/L305V/K337A/V158T-FVII,
F374Y/L305V/K337A/S314E-FVII, F374Y/L305V/V158D/E296V-FVII,
F374Y/L305V/V158D/M298Q-FVII, F374Y/L305V/V158D/S314E-FVII,
F374Y/L305V/E296V/M298Q-FVII, F374Y/L305V/E296V/V158T-FVII,
F374Y/L305V/E296V/S314E-FVII, F374Y/L305V/M298Q/V158T-FVII,
F374Y/L305V/M298Q/S314E-FVII, F374Y/L305V/V158T/S314E-FVII,
F374Y/K337A/S314E/V158T-FVII, F374Y/K337A/S314E/M298Q-FVII,
F374Y/K337A/S314E/E296V-FVII, F374Y/K337A/S314E/V158D-FVII,
F374Y/K337A/V158T/M298Q-FVII, F374Y/K337A/V158T/E296V-FVII,
F374Y/K337A/M298Q/E296V-FVII, F374Y/K337A/M298Q/V158D-FVII,
F374Y/K337A/E296V/V158D-FVII, F374Y/V158D/S314E/M298Q-FVII,
F374Y/V158D/S314E/E296V-FVII, F374Y/V158D/M298Q/E296V-FVII,
F374Y/V158T/S314E/E296V-FVII, F374Y/V158T/S314E/M298Q-FVII,
F374Y/V158T/M298Q/E296V-FVII, F374Y/E296V/S314E/M298Q-FVII,
F374Y/L305V/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/K337A/S314E-FVII,
F374Y/E296V/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/M298Q/K337A –FVII,
F374Y/L305V/E296V/M298Q/S314E-FVII, F374Y/V158D/E296V/M298Q/K337A-FVII,
F374Y/V158D/E296V/M298Q/S314E-FVII, F374Y/L305V/V158D/K337A/S314E-FVII,
F374Y/V158D/M298Q/K337A/S314E-FVII, F374Y/V158D/E296V/K337A/S314E-FVII,
F374Y/L305V/V158D/E296V/M298Q-FVII, F374Y/L305V/V158D/M298Q/K337A-FVII,
F374Y/L305V/V158D/E296V/K337A-FVII, F374Y/L305V/V158D/M298Q/S314E-FVII,
F374Y/L305V/V158D/E296V/S314E-FVII, F374Y/V158T/E296V/M298Q/K337A-FVII,
F374Y/V158T/E296V/M298Q/S314E-FVII, F374Y/L305V/V158T/K337A/S314E-FVII,
F374Y/V158T/M298Q/K337A/S314E-FVII, F374Y/V158T/E296V/K337A/S314E-FVII,
F374Y/L305V/V158T/E296V/M298Q-FVII, F374Y/L305V/V158T/M298Q/K337A-FVII,
F374Y/L305V/V158T/E296V/K337A-FVII, F374Y/L305V/V158T/M298Q/S314E-FVII,
F374Y/L305V/V158T/E296V/S314E-FVII, F374Y/E296V/M298Q/K337A/V158T/S314E-FVII,
F374Y/V158D/E296V/M298Q/K337A/S314E-FVII,
F374Y/L305V/V158D/E296V/M298Q/S314E-FVII,
F374Y/L305V/E296V/M298Q/V158T/S314E-FVII,
F374Y/L305V/E296V/M298Q/K337A/V158T-FVII,
F374Y/L305V/E296V/K337A/V158T/S314E-FVII,
F374Y/L305V/M298Q/K337A/V158T/S314E-FVII,
F374Y/L305V/V158D/E296V/M298Q/K337A-FVII,
F374Y/L305V/V158D/E296V/K337A/S314E-FVII,
F374Y/L305V/V158D/M298Q/K337A/S314E-FVII,
F374Y/L305V/E296V/M298Q/K337A/V158T/S314E-FVII,

F374Y/L305V/V158D/E296V/M298Q/K337A/S314E-FVII, S52A-Factor VII, S60A-Factor VII;
R152E-Factor VII, S344A-Factor VII, Factor VIIa lacking the Gla domain; and P11Q/K33E-FVII, T106N-FVII, K143N/N145T-FVII, V253N-FVII, R290N/A292T-FVII, G291N-FVII, R315N/V317T-FVII, K143N/N145T/R315N/V317T-FVII; and FVII having substitutions, additions or deletions in the amino acid sequence from 233Thr to 240Asn, FVII having substitutions, additions or deletions in the amino acid sequence from 304Arg to 329Cys.

The terminology for amino acid substitutions used are as follows. The first letter represent the amino acid naturally present at a position of human wild type FVII. The following number represent the position in human wild type FVII. The second letter represent the different amino acid substituting for (replacing) the natural amino acid. An example is M298Q, where an methionine at position 298 of human wild type FVII is replaced by a glutamine. In another example, V158T/M298Q, the valine in position 158 of human wild type FVII is replaced by a threonine and the methionine in position 298 of human wild type FVII is replaced by a Glutamine in the same Factor VII polypeptide.

In a further embodiment of the invention, the Factor VII related polypeptide polypeptide is a polypeptide, wherein the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 1.25. In one embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 2.0. In a further embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 4.0.

In a further embodiment of the invention, the Factor VII related polypeptide is a polypeptide, wherein the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 1.25 when tested in a Factor VIIa activity assay. In one embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 2.0 when tested in a Factor VIIa activity assay. In a further embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 4.0 when tested in a Factor VIIa activity assay. The Factor VIIa activity may be measured by the assays described in example 1.

In a further embodiment of the invention, the Factor VII related polypeptide is a polypeptide, wherein the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 1.25 when tested in the "*In Vitro* Hydrolysis Assay". In one embodiment the ratio between the activity of the

Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 2.0 when tested in the "*In Vitro* Hydrolysis Assay". In a further embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 4.0 when tested in the "*In Vitro* Hydrolysis Assay".

In a further embodiment of the invention, the Factor VII related polypeptide is a polypeptide, wherein the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 1.25 when tested in the "*In Vitro* Proteolysis Assay". In one embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 2.0 when tested in the "*In Vitro* Proteolysis Assay". In a further embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 4.0 when tested in the "*In Vitro* Proteolysis Assay". In a further embodiment the ratio between the activity of the Factor VII related polypeptide and the activity of the wild type human Factor VIIa is at least about 8.0 when tested in the "*In Vitro* Proteolysis Assay".

The present invention is suitable for Factor VII/VIIa variants with increased activity compared to wild type human Factor VIIa. Factor VII/VIIa variants with increased activity may be found by testing in suitable assays described in the following. These assays can be performed as a simple preliminary in vitro test. Thus, the example 1 discloses a simple test (entitled "In Vitro Hydrolysis Assay") for the activity of Factor VIIa variants of the invention. Based thereon, Factor VIIa variants which are of particular interest are such variants where the ratio between the activity of the variant and the activity of wild type human Factor VII is above 1.0, e.g. at least about 1.25, preferably at least about 2.0, such as at least about 3.0 or, even more preferred, at least about 4.0 when tested in the "In Vitro Hydrolysis Assay".

The activity of the variants can also be measured using a physiological substrate such as factor X ("In Vitro Proteolysis Assay") (see example 1), suitably at a concentration of 100-1000 nM, where the factor Xa generated is measured after the addition of a suitable chromogenic substrate (eg. S-2765). In addition, the activity assay may be run at physiological temperature.

The ability of the Factor VIIa variants to generate thrombin can also be measured in an assay comprising all relevant coagulation factors and inhibitors at physiological concen-trations (minus factor VIII when mimicking hemophilia A conditions) and activated platelets (as described on p. 543 in Monroe et al. (1997) Brit. J. Haematol. 99, 542-547 which is hereby incorporated as reference).

The term "identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or between polypeptides, as the case may be, as determined by the number of matches between strings of two or more nucleotide residues or two or more amino acid residues. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms").

The term "similarity" is a related concept, but in contrast to "identity", refers to a sequence relationship that includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, (fraction (10/20)) identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If, in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% ((fraction (15/20))). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates or other materials (i.e., contaminants) with which it is naturally associated, (2) is not covalently linked to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked covalently to a polypeptide to which it is not covalently linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment which would interfere with its therapeutic, diagnostic, prophylactic or research use.

Conservative modifications to the amino acid sequence of wild type human FVII (and the corresponding modifications to the encoding nucleotides) will produce FVII polypeptides having functional and chemical characteristics similar to those of naturally occurring FVII polypeptide. In contrast, substantial modifications in the functional and/or chemical characteristics of FVII polypeptides may be accomplished by selecting substitutions in the amino acid sequence of wild type human FVII that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (see, for example, MacLennan et al., 1998, Acta Physiol. Scand. Suppl. 643:55-67; Sasaki et al., 1998, Adv. Biophys. 35:1-24, which discuss alanine scanning mutagenesis).

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the Factor VII polypeptide, or to increase or decrease the affinity of the Factor VII polypeptides described herein.

Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human Factor VII polypeptide that are homologous with non-human Factor VII polypeptides, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., J. Mol. Biol., 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino

acids whose hydropathic indices are within .±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine ('3.0); aspartate (+3.0±1); glutamate (+3.0±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5±1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in wild type human FVII using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a Factor VII polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a Factor VII polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of the Factor VII polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a Factor VII polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of Factor VII polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a Factor VII polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays as described herein. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996), Chou et al., Biochemistry, 13(2):222-245 (1974); Chou et al., Biochemistry, 113(2):211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol, 47:45-148 (1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and

that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-9 (1996)), "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzymol., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Home, supra, and Brenner, supra).

Identity and similarity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., SIAM J. Applied Math., 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res., 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol., 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra). The well known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, Wis.), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino

acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3.times. the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually (fraction (1/10)) times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff et al., Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff et al., Proc. Natl. Acad. Sci USA, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following:

Algorithm: Needleman et al., J. Mol. Biol, 48:443-453 (1970); Comparison matrix: BLOSUM 62 from Henikoff et al., Proc. Natl. Acad. Sci. USA, 89:10915-10919 (1992); Gap Penalty: 12, Gap Length Penalty: 4, Threshold of Similarity: 0.

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparisons include the following: Algorithm: Needleman et al., J. Mol Biol., 48:443-453 (1970); Comparison matrix: matches=+10, mismatch=0, Gap Penalty: 50, Gap Length Penalty: 3.

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used,, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

Factor VII variants having a substantially modified biological activity relative to wild-type Factor VII include, without limitation, Factor VII variants that exhibit TF-independent Factor X proteolytic activity.

As described above the present invention further relates to a method for the treatment of bleeding episodes in a subject or for the enhancement of the normal haemostatic system, the method comprising administering to a subject in need thereof a

therapeutically or prophylactically effective amount of a first composition comprising wild type human FVIIa and a second composition comprising a Factor VII related polypeptide.

It is to be understood, that the subject is treated for the same bleeding or series of bleedings. In one embodiment the composition comprising wild type human FVIIa is administered first. In another embodiment the composition comprising a Factor VII related polypeptide is administered first.

The administration of the first composition comprising wild type human FVIIa and a second composition comprising a Factor VII related polypeptide may be simultaneous or near simultanous. Alternatively the first composition comprising wild type human FVIIa and the second composition comprising a Factor VII related polypeptide may be administered in such a way that the first and second composition is administered to the subject separated by a certain amont of time. This amount of time separating the administration of the first composition comprising wild type human FVIIa and second composition comprising a Factor VII related polypeptide may be from about 1 minute to about 24 hours, such as from about 5 min to about 20 hours, such as from about 10 minutes to about 10 hours, such as from about 10 min to about 5 hours, such as from about 10 minutes to about 2 hours. In one embodiment the time separating the administration of the first composition comprising wild type human FVIIa and second composition comprising a Factor VII related polypeptide is from about 1 minute to about 10 minutes.

The term "enhancement of the normal haemostatic system" means an enhancement of the ability to generate thrombin.

As used herein the term "bleeding disorder" reflects any defect, congenital, acquired or induced, of cellular or molecular origin that is manifested in bleedings. Examples are clotting factor deficiencies (e.g. haemophilia A and B or deficiency of coagulation Factors XI or VII), clotting factor inhibitors, defective platelet function, thrombocytopenia or von Willebrand's disease.

The term "bleeding episodes" is meant to include uncontrolled and excessive bleeding which is a major problem both in connection with surgery and other forms of tissue damage. Uncontrolled and excessive bleeding may occur in subjects having a normal coagulation system and subjects having coagulation or bleeding disorders. Clotting factor deficiencies (haemophilia A and B, deficiency of coagulation factors XI or VII) or clotting factor inhibitors may be the cause of bleeding disorders. Excessive bleedings also occur in subjects with a normally functioning blood clotting cascade (no clotting factor deficiencies or -inhibitors against any of the coagulation factors) and may be caused by a defective platelet function, thrombocytopenia or von Willebrand's disease. In such cases, the bleedings may be likened to those bleedings caused by haemophilia

because the haemostatic system, as in haemophilia, lacks or has abnormal essential clotting "compounds" (such as platelets or von Willebrand factor protein) that causes major bleedings. In subjects who experience extensive tissue damage in association with surgery or vast trauma, the normal haemostatic mechanism may be overwhelmed by the demand of immediate haemostasis and they may develop bleeding in spite of a normal haemostatic mechanism. Achieving satisfactory haemostasis also is a problem when bleedings occur in organs such as the brain, inner ear region and eyes with limited possibility for surgical haemostasis. The same problem may arise in the process of taking biopsies from various organs (liver, lung, tumour tissue, gastrointestinal tract) as well as in laparoscopic surgery. Common for all these situations is the difficulty to provide haemostasis by surgical techniques (sutures, clips, etc.) which also is the case when bleeding is diffuse (haemorrhagic gastritis and profuse uterine bleeding). Acute and profuse bleedings may also occur in subjects on anticoagulant therapy in whom a defective haemostasis has been induced by the therapy given. Such subjects may need surgical interventions in case the anticoagulant effect has to be counteracted rapidly. Radical retropubic prostatectomy is a commonly performed procedure for subjects with localized prostate cancer. The operation is frequently complicated by sigrnificant and sometimes massive blood loss. The considerable blood loss during prostatectomy is mainly related to the complicated anatomical situation, with various densely vascularized sites that are not easily accessible for surgical haemostasis, and which may result in diffuse bleeding from a large area. Another situation that may cause problems in the case of unsatisfactory haemostasis is when subjects with a normal haemostatic mechanism are given anticoagulant therapy to prevent thromboembolic disease. Such therapy may include heparin, other forms of proteoglycans, warfarin or other forms of vitamin K-antagonists as well as aspirin and other platelet aggregation inhibitors.

In one embodiment of the invention, the bleeding is associated with haemophilia. In another embodiment, the bleeding is associated with haemophilia with aquired inhibitors. In another embodiment, the bleeding is associated with thrombocytopenia. In another embodiment, the bleeding is associated with von Willebrand's disease. In another embodiment, the bleeding is associated with severe tissue damage. In another embodiment, the bleeding is associated with severe trauma. In another embodiment, the bleeding is associated with surgery. In another embodiment, the bleeding is associated with laparoscopic surgery. In another embodiment, the bleeding is associated with haemorrhagic gastritis. In another embodiment, the bleeding is profuse uterine bleeding. In another embodiment, the bleeding is occurring in organs with a limited possibility for mechanical haemostasis. In another embodiment, the bleeding is occurring in the brain, inner ear region or eyes. In another embodiment, the

bleeding is associated with the process of taking biopsies. In another embodiment, the bleeding is associated with anticoagulant therapy.

The term "subject" as used herein is intended to mean any animal, in particular mammals, such as humans, and may, where appropriate, be used interchangeably with the term "patient".

The Factor VII polypeptides described herein may be produced by means of recombinant nucleic acid techniques. In general, a cloned wild-type Factor VII nucleic acid sequence is modified to encode the desired protein. This modified sequence is then inserted into an expression vector, which is in turn transformed or transfected into host cells. Higher eukaryotic cells, in particular cultured mammalian cells, are preferred as host cells. The complete nucleotide and amino acid sequences for human Factor VII are known (see U.S. 4,784,950, where the cloning and expression of recombinant human Factor VII is described). The bovine Factor VII sequence is described in Takeya et al., J. Biol. Chem. 263:14868-14872 (1988)).

The amino acid sequence alterations may be accomplished by a variety of techniques. Modification of the nucleic acid sequence may be by site-specific mutagenesis. Techniques for site-specific mutagenesis are well known in the art and are described in, for example, Zoller and Smith (DNA 3:479-488, 1984) or "Splicing by extension overlap", Horton et al., Gene 77, 1989, pp. 61-68. Thus, using the nucleotide and amino acid sequences of Factor VII, one may introduce the alteration(s) of choice. Likewise, procedures for preparing a DNA construct using polymerase chain reaction using specific primers are well known to persons skilled in the art (cf. PCR Protocols, 1990, Academic Press, San Diego, California, USA).

The nucleic acid construct encoding the Factor VII polypeptide of the invention may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the polypeptide by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. Ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

The nucleic acid construct encoding the Factor VII polypeptide may also be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by Beaucage and Caruthers, Tetrahedron Letters 22 (1981), 1859 - 1869, or the method described by Matthes et al., EMBO Journal 3 (1984), 801 - 805. According to the phosphoamidite method, oligonucleotides are synthesised, e.g. in an automatic DNA synthesiser, purified, annealed, ligated and cloned in suitable vectors.

Furthermore, the nucleic acid construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating

fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire nucleic acid construct, in accordance with standard techniques.

The nucleic acid construct is preferably a DNA construct. DNA sequences for use in producing Factor VII polypeptides according to the present invention will typically encode a pre-pro polypeptide at the amino-terminus of Factor VII to obtain proper posttranslational processing (e.g. gamma-carboxylation of glutamic acid residues) and secretion from the host cell. The pre-pro polypeptide may be that of Factor VII or another vitamin K-dependent plasma protein, such as Factor IX, Factor X, prothrombin, protein C or protein S. As will be appreciated by those skilled in the art, additional modifications can be made in the amino acid sequence of the Factor VII polypeptides where those modifications do not significantly impair the ability of the protein to act as a coagulant. For example, the Factor VII polypeptides can also be modified in the activation cleavage site to inhibit the conversion of zymogen Factor VII into its activated two-chain form, as generally described in U.S. 5,288,629.

Expression vectors for use in expressing Factor VIIa variants will comprise a promoter capable of directing the transcription of a cloned gene or cDNA. Preferred promoters for use in cultured mammalian cells include viral promoters and cellular promoters. Viral promoters include the SV40 promoter (Subramani et al., Mol. Cell. Biol. 1:854-864, 1981) and the CMV promoter (Boshart et al., Cell 41:521-530, 1985). A particularly preferred viral promoter is the major late promoter from adenovirus 2 (Kaufman and Sharp, Mol. Cell. Biol. 2:1304-1319, 1982). Cellular promoters include the mouse kappa gene promoter (Bergman et al., Proc. Natl. Acad. Sci. USA 81:7041-7045, 1983) and the mouse VH promoter (Loh et al., Cell 33:85-93, 1983). A particularly preferred cellular promoter is the mouse metallothionein-I promoter (Palmiter et al., Science 222:809-814, 1983). Expression vectors may also contain a set of RNA splice sites located downstream from the promoter and upstream from the insertion site for the Factor VII sequence itself. Preferred RNA splice sites may be obtained from adenovirus and/or immunoglobulin genes. Also contained in the expression vectors is a polyadenylation signal located downstream of the insertion site. Particularly preferred polyadenylation signals include the early or late polyadenylation signal from SV40 (Kaufman and Sharp, ibid.), the polyadenylation signal from the adenovirus 5 Elb region, the human growth hormone gene terminator (DeNoto et al. Nucl. Acids Res. 9:3719-3730, 1981) or the polyadenylation signal from the human Factor VII gene or the bovine Factor VII gene. The expression vectors may also include a noncoding viral leader sequence, such as the adenovirus 2 tripartite leader, located between the promoter and the RNA splice sites; and enhancer sequences, such as the SV40 enhancer.

Cloned DNA sequences are introduced into cultured mammalian cells by, for example, calcium phosphate-mediated transfection (Wigler et al., Cell 14:725-732, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603-616, 1981; Graham and Van der Eb, Virology 52d:456-467, 1973) or electroporation (Neumann et al., EMBO J. 1:841-845, 1982). To identify and select cells that express the exogenous DNA, a gene that confers a selectable phenotype (a selectable marker) is generally introduced into cells along with the gene or cDNA of interest. Preferred selectable markers include genes that confer resistance to drugs such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. A preferred amplifiable selectable marker is a dihydrofolate reductase (DHFR) sequence. Selectable markers are reviewed by Thilly (Mammalian Cell Technology, Butterworth Publishers, Stoneham, MA, incorporated herein by reference). The person skilled in the art will easily be able to choose suitable selectable markers.

Selectable markers may be introduced into the cell on a separate plasmid at the same time as the gene of interest, or they may be introduced on the same plasmid. If, on the same plasmid, the selectable marker and the gene of interest may be under the control of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, Levinson and Simonsen, U.S. 4,713,339). It may also be advantageous to add additional DNA, known as "carrier DNA," to the mixture that is introduced into the cells. After the cells have taken up the DNA, they are grown in an appropriate growth medium, typically for 1-2 days, to begin expressing the gene of interest. The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media are prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, More Gene Manipulations in Fungi, Academic Press, CA, 1991). Growth media generally include a carbon source, a nitrogen source, essential amino acids, essential sugars, vitamins, salts, phospholipids, proteins and growth factors. For production of gamma-carboxylated Factor VII polypeptides, the medium will contain vitamin K, preferably at a concentration of about 0.1 mg/ml to about 5 mg/ml. Drug selection is then applied to select for the growth of cells that are expressing the selectable marker in a stable fashion. For cells that have been transfected with an amplifiable selectable marker the drug concentration may be increased to select for an increased copy number of the cloned sequences, thereby increasing expression levels.

Clones of stably transfected cells are then screened for expression of the desired Factor VII polypeptide.

Preferred mammalian cell lines include the CHO (ATCC CCL 61), COS-1 (ATCC CRL 1650), baby hamster kidney (BHK) and 293 (ATCC CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) cell lines. A preferred BHK cell line is the tk- ts13 BHK cell line (Waechter and Baserga, Proc. Natl. Acad. Sci. USA 79:1106-1110, 1982), hereinafter referred to as BHK 570 cells. The BHK 570 cell line is available from the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852, under ATCC accession number CRL 10314. A tk- ts13 BHK cell line is also available from the ATCC under accession number CRL 1632. In addition, a number of other cell lines may be used, including Rat Hep I (Rat hepatoma; ATCC CRL 1600), Rat Hep II (Rat hepatoma; ATCC CRL 1548), TCMK (ATCC CCL 139), Human lung (ATCC HB 8065), NCTC 1469 (ATCC CCL 9.1) and DUKX cells (Urlaub and Chasin, Proc. Natl. Acad. Sci. USA 77:4216-4220, 1980).

Transgenic animal technology may be employed to produce the Factor VII polypeptides of the invention. It is preferred to produce the proteins within the mammary glands of a host female mammal. Expression in the mammary gland and subsequent secretion of the protein of interest into the milk overcomes many difficulties encountered in isolating proteins from other sources. Milk is readily collected, available in large quantities, and biochemically well characterized. Furthermore, the major milk proteins are present in milk at high concentrations (typically from about 1 to 15 g/l).

From a commercial point of view, it is clearly preferable to use as the host a species that has a large milk yield. While smaller animals such as mice and rats can be used (and are preferred at the proof of principle stage), it is preferred to use livestock mammals including, but not limited to, pigs, goats, sheep and cattle. Sheep are particularly preferred due to such factors as the previous history of transgenesis in this species, milk yield, cost and the ready availability of equipment for collecting sheep milk (see, for example, WO 88/00239 for a comparison of factors influencing the choice of host species). It is generally desirable to select a breed of host animal that has been bred for dairy use, such as East Friesland sheep, or to introduce dairy stock by breeding of the transgenic line at a later date. In any event, animals of known, good health status should be used.

To obtain expression in the mammary gland, a transcription promoter from a milk protein gene is used. Milk protein genes include those genes encoding caseins (see U.S. 5,304,489), beta lactoglobulin, a lactalbumin, and whey acidic protein. The beta lactoglobulin (BLG) promoter is preferred. In the case of the ovine beta lactoglobulin gene, a region of at least the proximal 406 bp of 5' flanking sequence of the gene will

generally be used, although larger portions of the 5' flanking sequence, up to about 5 kbp, are preferred, such as a ~4.25 kbp DNA segment encompassing the 5' flanking promoter and non coding portion of the beta lactoglobulin gene (see Whitelaw et al., Biochem. J. 286: 31 39 (1992)). Similar fragments of promoter DNA from other species are also suitable.

Other regions of the beta lactoglobulin gene may also be incorporated in constructs, as may genomic regions of the gene to be expressed. It is generally accepted in the art that constructs lacking introns, for example, express poorly in comparison with those that contain such DNA sequences (see Brinster et al., Proc. Natl. Acad. Sci. USA 85: 836 840 (1988); Palmiter et al., Proc. Natl. Acad. Sci. USA 88: 478 482 (1991); Whitelaw et al., Transgenic Res. 1: 3 13 (1991); WO 89/01343; and WO 91/02318, each of which is incorporated herein by reference). In this regard, it is generally preferred, where possible, to use genomic sequences containing all or some of the native introns of a gene encoding the protein or polypeptide of interest, thus the further inclusion of at least some introns from, e.g, the beta lactoglobulin gene, is preferred. One such region is a DNA segment that provides for intron splicing and RNA polyadenylation from the 3' non coding region of the ovine beta lactoglobulin gene. When substituted for the natural 3' non coding sequences of a gene, this ovine beta lactoglobulin segment can both enhance and stabilize expression levels of the protein or polypeptide of interest. Within other embodiments, the region surrounding the initiation ATG of the variant Factor VII sequence is replaced with corresponding sequences from a milk specific protein gene. Such replacement provides a putative tissue specific initiation environment to enhance expression. It is convenient to replace the entire variant Factor VII pre pro and 5' non coding sequences with those of, for example, the BLG gene, although smaller regions may be replaced.

For expression of Factor VII polypeptides in transgenic animals, a DNA segment encoding variant Factor VII is operably linked to additional DNA segments required for its expression to produce expression units. Such additional segments include the above mentioned promoter, as well as sequences that provide for termination of transcription and polyadenylation of mRNA. The expression units will further include a DNA segment encoding a secretory signal sequence operably linked to the segment encoding modified Factor VII. The secretory signal sequence may be a native Factor VII secretory signal sequence or may be that of another protein, such as a milk protein (see, for example, von Heijne, Nucl. Acids Res. 14: 4683 4690 (1986); and Meade et al., U.S. 4,873,316, which are incorporated herein by reference).

Construction of expression units for use in transgenic animals is conveniently carried out by inserting a variant Factor VII sequence into a plasmid or phage vector

containing the additional DNA segments, although the expression unit may be constructed by essentially any sequence of ligations. It is particularly convenient to provide a vector containing a DNA segment encoding a milk protein and to replace the coding sequence for the milk protein with that of a variant Factor VII polypeptide; thereby creating a gene fusion that includes the expression control sequences of the milk protein gene. In any event, cloning of the expression units in plasmids or other vectors facilitates the amplification of the variant Factor VII sequence. Amplification is conveniently carried out in bacterial (e.g. E. coli) host cells, thus the vectors will typically include an origin of replication and a selectable marker functional in bacterial host cells. The expression unit is then introduced into fertilized eggs (including early stage embryos) of the chosen host species. Introduction of heterologous DNA can be accomplished by one of several routes, including microinjection (e.g. U.S. Patent No. 4,873,191), retroviral infection (Jaenisch, Science 240: 1468 1474 (1988)) or site directed integration using embryonic stem (ES) cells (reviewed by Bradley et al., Bio/Technology 10: 534 539 (1992)). The eggs are then implanted into the oviducts or uteri of pseudopregnant females and allowed to develop to term. Offspring carrying the introduced DNA in their germ line can pass the DNA on to their progeny in the normal, Mendelian fashion, allowing the development of transgenic herds. General procedures for producing transgenic animals are known in the art (see, for example, Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, 1986; Simons et al., Bio/Technology 6: 179 183 (1988); Wall et al., Biol. Reprod. 32: 645 651 (1985); Buhler et al., Bio/Technology 8: 140 143 (1990); Ebert et al., Bio/Technology 9: 835 838 (1991); Krimpenfort et al., Bio/Technology 9: 844 847 (1991); Wall et al., J. Cell. Biochem. 49: 113 120 (1992); U.S. 4,873,191; U.S. 4,873,316; WO 88/00239, WO 90/05188, WO 92/11757; and GB 87/00458). Techniques for introducing foreign DNA sequences into mammals and their germ cells were originally developed in the mouse (see, e.g., Gordon et al., Proc. Natl. Acad. Sci. USA 77: 7380 7384 (1980); Gordon and Ruddle, Science 214: 1244 1246 (1981); Palmiter and Brinster, Cell 41: 343 345 (1985); Brinster et al., Proc. Natl. Acad. Sci. USA 82: 4438 4442 (1985); and Hogan et al. (ibid.)). These techniques were subsequently adapted for use with larger animals, including livestock species (see, e.g., WO 88/00239, WO 90/05188, and WO 92/11757; and Simons et al., Bio/Technology 6: 179 183 (1988)). To summarise, in the most efficient route used to date in the generation of transgenic mice or livestock, several hundred linear molecules of the DNA of interest are injected into one of the pro nuclei of a fertilized egg according to established techniques. Injection of DNA into the cytoplasm of a zygote can also be employed.

Production in transgenic plants may also be employed. Expression may be generalised or directed to a particular organ, such as a tuber (see, Hiatt, Nature 344:469 479 (1990); Edelbaum et al., J. Interferon Res. 12:449 453 (1992); Sijmons et al., Bio/Technology 8:217 221 (1990); and EP 0 255 378).

The Factor VII polypeptides of the invention are recovered from cell culture medium or milk. The Factor VII polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction (see, e.g., Protein Purification, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989). Preferably, they may be purified by affinity chromatography on an anti-Factor VII antibody column. The use of calcium-dependent monoclonal antibodies is described by Wakabayashi et al., J. Biol. Chem. 261:11097-11108, (1986) and Thim et al., Biochemistry 27: 7785-7793, (1988). Additional purification may be achieved by conventional chemical purification means, such as high performance liquid chromatography. Other methods of purification, including barium citrate precipitation, are known in the art, and may be applied to the purification of the novel Factor VII polypeptides described herein (see, for example, Scopes, R., Protein Purification, Springer-Verlag, N.Y., 1982).

For therapeutic purposes it is preferred that the Factor VII polypeptides of the invention are substantially pure. Thus, in a preferred embodiment of the invention the Factor VII polypeptides of the invention is purified to at least about 90 to 95% homogeneity, preferably to at least about 98% homogeneity. Purity may be assessed by e.g. gel electrophoresis and amino-terminal amino acid sequencing.

The Factor VII polypeptide is cleaved at its activation site in order to convert it to its two-chain form. Activation may be carried out according to procedures known in the art, such as those disclosed by Osterud, et al., Biochemistry 11:2853-2857 (1972); Thomas, U.S. Patent No. 4,456,591; Hedner and Kisiel, J. Clin. Invest. 71:1836-1841 (1983); or Kisiel and Fujikawa, Behring Inst. Mitt. 73:29-42 (1983). Alternatively, as described by Bjoern et al. (Research Disclosure, 269 September 1986, pp. 564-565), Factor VII may be activated by passing it through an ion-exchange chromatography column, such as Mono Q (Pharmacia fine Chemicals) or the like. The resulting activated Factor VII polypeptide may then be formulated and administered as described below.

Example 1

Assay 1:
In Vitro Hydrolysis Assay

Wild type (native) Factor VIIa and Factor VIIa variant (both hereafter referred to as "Factor VIIa") are assayed in parallel to directly compare their specific activities. The assay is carried out in a microtiter plate (MaxiSorp, Nunc, Denmark). The chromogenic substrate D-Ile-Pro-Arg-p-nitroanilide (S-2288, Chromogenix, Sweden), final concentration 1 mM, is added to Factor VIIa (final concentration 100 nM) in 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM $CaCl_2$ and 1 mg/ml bovine serum albumin. The absorbance at 405 nm is measured continuously in a SpectraMax® 340 plate reader (Molecular Devices, USA). The absorbance developed during a 20-minute incubation, after subtraction of the absorbance in a blank well containing no enzyme, is used to calculate the ratio between the activities of vari-ant and wild-type Factor VIIa:

Ratio = (A405 nm Factor VIIa variant)/(A405 nm Factor VIIa wild-type).

Assay 2:

In Vitro Proteolysis Assay

Wild type (native) Factor VIIa and Factor VIIa variant (both hereafter referred to as "Factor VIIa") are assayed in parallel to directly compare their specific activities. The assay is carried out in a microtiter plate (MaxiSorp, Nunc, Denmark). Factor VIIa (10 nM) and Factor X (0.8 microM) in 100 microL 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM $CaCl_2$ and 1 mg/ml bovine serum albumin, are incubated for 15 min. Factor X cleavage is then stopped by the addition of 50 microL 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 20 mM EDTA and 1 mg/ml bovine serum albumin. The amount of Factor Xa generated is measured by addition of the chromogenic substrate Z-D-Arg-Gly-Arg-p-nitroanilide (S-2765, Chromogenix, Sweden), final concentration 0.5 mM. The absorbance at 405 nm is measured continuously in a SpectraMax® 340 plate reader (Molecular Devices, USA). The absorbance developed dur-ing 10 minutes, after subtraction of the absorbance in a blank well containing no FVIIa, is used to calculate the ratio between the proteolytic activities of variant and wild-type Factor VIIa:

Ratio = (A405 nm Factor VIIa variant)/(A405 nm Factor VIIa wild-type).

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed, since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to

fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Example 2

Formulation of the following liquid, aqueous pharmaceutical compositions is envisaged:

A)	
rhFVIIa	0.9 mg/mL (approx. 50,000 IU/mL)
V158D/E296V/M298Q-FVIIa	0.1 mg/mL
PIPES	15.12 mg/mL (50 mM)
Poloxamer 188	1.0 mg/mL
Sodium chloride	2.92 mg/mL (50 mM)
Calcium chloride 2 H_2O	1.47 mg/mL (10 mM)
Methionine	0.5 mg/mL
1 M NaOH	added to pH 6.5
water for injection (WFI)	ad 2.0 mL
B)	
rhFVIIa	0.8 mg/mL (approx. 50,000 IU/mL)
V158D/E296V/M298Q-FVIIa	0.2 mg/mL
PIPES	15.12 mg/mL (50 mM)
Poloxamer 188	1.0 mg/mL
Sodium chloride	2.92 mg/mL (50 mM)
Calcium chloride 2 H_2O	1.47 mg/mL (10 mM)
Methionine	0.5 mg/mL
1 M NaOH	added to pH 6.5
water for injection (WFI)	ad 2.0 mL
C)	
rhFVIIa	0.9 mg/mL (approx. 50,000 IU/mL)
V158D/E296V/M298Q-FVIIa	0.1 mg/mL
PIPES	15.12 mg/mL (50 mM)
Poloxamer 188	1.0 mg/mL
Sodium chloride	2.92 mg/mL (50 mM)
Calcium chloride 2 H_2O	1.47 mg/mL (10 mM)
1 M NaOH	added to pH 6.5
water for injection (WFI)	ad 2.0 mL
D)	
rhFVIIa	0.8 mg/mL (approx. 50,000 IU/mL)
V158D/E296V/M298Q-FVIIa	0.2 mg/mL
PIPES	15.12 mg/mL (50 mM)
Poloxamer 188	1.0 mg/mL
Sodium chloride	2.92 mg/mL (50 mM)
Calcium chloride 2 H_2O	1.47 mg/mL (10 mM)
1 M NaOH	added to pH 6.5
water for injection (WFI)	ad 2.0 mL

Pharmaceutical compositions A-D can subsequently be transferred to sterile vials or cartridges flushed with nitrogen or argon, and can then be packed in air-tight aluminium-laminated plastic bags.

Example 3

Other pharmaceutical formulations is prepared by adding 10 mM histidine, 10 mM sodium acetate and 50 mM benzamidine (only for formulations E and F) to a mix of 0.9 mg/mL bulk solution of rFVIIa containing 0.1 mg/mL V158D/E296V/M298Q-FVIIa and already containing glycylglycine, sodium chloride and calcium chloride in the below mentioned concentrations. pH is finally adjusted to 6.5 and 7.0, respectively, with 1 M sodium hydroxide.

E)

0.9 mg/mL rhFVIIa
0.1 mg/mL V158D/E296V/M298Q-FVIIa
10 mM Histidine
10 mM Sodium acetate
10 mM Glycylglyclne
50 mM Sodium chloride
10 mM Calcium chloride
50 mM Benzamidine
pH = 6.5

F)

0.9 mg/mL rhFVIIa
0.1 mg/mL V158D/E296V/M298Q-FVIIa
10 mM Histidine
10 mM Sodium acetate
10 mM Glycylglycine
50 mM Sodium chloride
10 mM Calcium chloride
50 mM Benzamidine
pH = 7.0

G)

0.9 mg/mL rhFVIIa
0.1 mg/mL V158D/E296V/M298Q-FVIIa
10 mM Sodium acetate

10 mM Glycylglycine
50 mM Sodium chloride
10 mM Calcium chloride
10 mM Histidine
pH = 6.5

H)

0.9 mg/mL rhFVIIa
0.1 mg/mL V158D/E296V/M298Q-FVIIa
10 mM Histidine
10 mM Sodium acetate
10 mM Glycylglycine
50 mM Sodium chloride
10 mM Calcium chloride
pH = 7.0

Example 4

Typical compositions comprising wild type human FVIIa and a Factor VII related polypeptide are shown. Typical excipients and their typical amounts are shown.

Table 1 shows the concentration of active ingredients and excipients in the event where the composition is in liquid form, that is, in the composition before finalising the manufacturing (e.g. finalising the freeze-drying), or in the reconstituted solution.

Table 2 shows the concentration of active ingredient and excipients in the event where the composition is in solid form, i.e. in freeze-dried form.

Table 1. Compositions, content of excipients in solution.

Main Function:	Excipients:	Content (mg/ml) in liquid composition
Active Ingredient	rhFVIIa	0.6 - 5
Active Ingredient	V158D/E296V/M298Q-FVIIa	0.6 - 5
Tonicity modifier	Sodium Chloride	0 -4
Tonicity modifier/ stabiliser	Calcium Chloride, $2H_20$	1 - 7
Buffering agent	Glycylglycine	1.32 (0-1.5)
Surfactant	Polysorbate 80	0.05 - 2

Bulking Agent/ Cryoprotectant/ Lyoprotectant	Mannitol	10 - 40
Bulking Agent/ Cryoprotectant/ Lyoprotectant	Sucrose	10 - 40
Antioxidant	Methionine	0 - 1.0
	pH	5-6

Table 2. Compositions, content of excipients in freeze-dried form.

Main Function:	Excipients:	Content (% w/w) in solid composition
Active Ingredient	rhFVIIa	0.6 – 19
Active Ingredient	V158D/E296V/M298Q-FVIIa	0.6 – 19
Tonicity modifier	Sodium Chloride	0 – 15
Tonicity modifier/ stabiliser	Calcium Chloride, $2H_20$	1.0 to 24.0
Buffering agent	Glycylglycine	0 - 6.0
Surfactant	Polysorbate 80	0.05 - 8.0
Bulking Agent/ Cryoprotectant/ Lyoprotectant	Mannitol	13 – 76
Bulking Agent/ Cryoprotetant/ Lyoprotectant	Sucrose	13 - 76
Antioxidant	Methionine	0 – 4.2
	pH	5 - 6

Example 5

Human wild type Factor VIIa may be administered to a patient as a single dose comprising a single-dose-effective amount for treating of of bleeding episodes, e.g. trauma, or in a staged series of doses which together comprise an effective amount for treating of bleeding episodes, e.g. trauma. An effective amount of human wild type Factor VIIa refers to the amount of human wild type Factor VIIa which, when administered in a single dose or in the aggregate of multiple doses, or as part of any

other type of defined treatment regimen, produces a measurable improvement in at least one clinical parameter associated with trauma.

Administration of a single dose refers to administration of an entire dose of human wild type Factor VIIa as a bolus over a period of less than about 5 minutes. In some embodiments, the administration occurs over a period of less than about 2.5 minutes, and, in some, over less than about 1 min. Typically, a single-dose effective amount comprises at least about 40 ug/kg human Factor VIIa or a corresponding amount of a Factor VIIa equivalent, such as, at least about 50 ug/kg, 75 ug/kg, or 90 ug/kg, or at least 150 ug/kg Factor VIIa.

In practicing one aspect of the present invention, where an effective amount of human wild type Factor VIIa is not achived, a Factor VII related polypeptide, such as V158D/E296V/M298Q-FVIIa is administered subsequently to the administration of human wild type Factor VIIa.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The amino acid sequence of wild type human coagulation Factor VII.

CLAIMS

1. A composition comprising wild type human FVIIa and a Factor VII related polypeptide.

2. The composition according to claim 1, wherein the molar ratio between said wild type human FVIIa and said Factor VII related polypeptide is from about 1:99 to about 99:1

3. The composition according to any one of claims 1 or 2, wherein said Factor VII related polypeptide has a proteolytic activity higher than wild type human FVIIa.

4. The composition according to any one of claims 1-3, wherein said Factor VII related polypeptide is selected from the group consisting of: L305V-FVII, L305V/M306D/D309S-FVII, L305I-FVII, L305T-FVII, F374P-FVII, V158T/M298Q-FVII, V158D/E296V/M298Q-FVII, K337A-FVII, M298Q-FVII, V158D/M298Q-FVII, L305V/K337A-FVII, V158D/E296V/M298Q/L305V-FVII, V158D/E296V/M298Q/K337A-FVII, V158D/E296V/M298Q/L305V/K337A-FVII, K157A-FVII, E296V-FVII, E296V/M298Q-FVII, V158D/E296V-FVII, V158D/M298K-FVII, and S336G-FVII, L305V/K337A-FVII, L305V/V158D-FVII, L305V/E296V-FVII, L305V/M298Q-FVII, L305V/V158T-FVII, L305V/K337A/V158T-FVII, L305V/K337A/M298Q-FVII, L305V/K337A/E296V-FVII, L305V/K337A/V158D-FVII, L305V/V158D/M298Q-FVII, L305V/V158D/E296V-FVII, L305V/V158T/M298Q-FVII, L305V/V158T/E296V-FVII, L305V/E296V/M298Q-FVII, L305V/V158D/E296V/M298Q-FVII, L305V/V158T/E296V/M298Q-FVII, L305V/V158T/K337A/M298Q-FVII, L305V/V158T/E296V/K337A-FVII, L305V/V158D/K337A/M298Q-FVII, L305V/V158D/E296V/K337A–FVII, L305V/V158D/E296V/M298Q/K337A-FVII, L305V/V158T/E296V/M298Q/K337A-FVII, S314E/K316H-FVII, S314E/K316Q-FVII, S314E/L305V-FVII, S314E/K337A-FVII, S314E/V158D-FVII, S314E/E296V-FVII, S314E/M298Q-FVII, S314E/V158T-FVII, K316H/L305V-FVII, K316H/K337A-FVII, K316H/V158D-FVII, K316H/E296V-FVII, K316H/M298Q-FVII, K316H/V158T-FVII, K316Q/L305V-FVII, K316Q/K337A-FVII, K316Q/V158D-FVII, K316Q/E296V-FVII, K316Q/M298Q-FVII, K316Q/V158T-FVII, S314E/L305V/K337A-FVII, S314E/L305V/V158D-FVII, S314E/L305V/E296V-FVII, S314E/L305V/M298Q-FVII, S314E/L305V/V158T-FVII, S314E/L305V/K337A/V158T-FVII, S314E/L305V/K337A/M298Q-FVII, S314E/L305V/K337A/E296V-FVII, S314E/L305V/K337A/V158D-FVII, S314E/L305V/V158D/M298Q-FVII, S314E/L305V/V158D/E296V-FVII, S314E/L305V/V158T/M298Q-FVII, S314E/L305V/V158T/E296V-FVII, S314E/L305V/E296V/M298Q-FVII, S314E/L305V/V158D/E296V/M298Q-FVII, S314E/L305V/V158T/E296V/M298Q-FVII,

S314E/L305V/V158T/K337A/M298Q-FVII, S314E/L305V/V158T/E296V/K337A-FVII, S314E/L305V/V158D/K337A/M298Q-FVII, S314E/L305V/V158D/E296V/K337A-FVII, S314E/L305V/V158D/E296V/M298Q/K337A-FVII, S314E/L305V/V158T/E296V/M298Q/K337A-FVII, K316H/L305V/K337A-FVII, K316H/L305V/V158D-FVII, K316H/L305V/E296V-FVII, K316H/L305V/M298Q-FVII, K316H/L305V/V158T-FVII, K316H/L305V/K337A/V158T-FVII, K316H/L305V/K337A/M298Q-FVII, K316H/L305V/K337A/E296V-FVII, K316H/L305V/K337A/V158D-FVII, K316H/L305V/V158D/M298Q-FVII, K316H/L305V/V158D/E296V-FVII, K316H/L305V/V158T/M298Q-FVII, K316H/L305V/V158T/E296V-FVII, K316H/L305V/E296V/M298Q-FVII, K316H/L305V/V158D/E296V/M298Q-FVII, K316H/L305V/V158T/E296V/M298Q-FVII, K316H/L305V/V158T/K337A/M298Q-FVII, K316H/L305V/V158T/E296V/K337A-FVII, K316H/L305V/V158D/K337A/M298Q-FVII, K316H/L305V/V158D/E296V/K337A –FVII, K316H/L305V/V158D/E296V/M298Q/K337A-FVII, K316H/L305V/V158T/E296V/M298Q/K337A-FVII, K316Q/L305V/K337A-FVII, K316Q/L305V/V158D-FVII, K316Q/L305V/E296V-FVII, K316Q/L305V/M298Q-FVII, K316Q/L305V/V158T-FVII, K316Q/L305V/K337A/V158T-FVII, K316Q/L305V/K337A/M298Q-FVII, K316Q/L305V/K337A/E296V-FVII, K316Q/L305V/K337A/V158D-FVII, K316Q/L305V/V158D/M298Q-FVII, K316Q/L305V/V158D/E296V-FVII, K316Q/L305V/V158T/M298Q-FVII, K316Q/L305V/V158T/E296V-FVII, K316Q/L305V/E296V/M298Q-FVII, K316Q/L305V/V158D/E296V/M298Q-FVII, K316Q/L305V/V158T/E296V/M298Q-FVII, K316Q/L305V/V158T/K337A/M298Q-FVII, K316Q/L305V/V158T/E296V/K337A-FVII, K316Q/L305V/V158D/K337A/M298Q-FVII, K316Q/L305V/V158D/E296V/K337A –FVII, K316Q/L305V/V158D/E296V/M298Q/K337A-FVII, K316Q/L305V/V158T/E296V/M298Q/K337A-FVII, F374Y/K337A-FVII, F374Y/V158D-FVII, F374Y/E296V-FVII, F374Y/M298Q-FVII, F374Y/V158T-FVII, F374Y/S314E-FVII, F374Y/L305V-FVII, F374Y/L305V/K337A-FVII, F374Y/L305V/V158D-FVII, F374Y/L305V/E296V-FVII, F374Y/L305V/M298Q-FVII, F374Y/L305V/V158T-FVII, F374Y/L305V/S314E-FVII, F374Y/K337A/S314E-FVII, F374Y/K337A/V158T-FVII, F374Y/K337A/M298Q-FVII, F374Y/K337A/E296V-FVII, F374Y/K337A/V158D-FVII, F374Y/V158D/S314E-FVII, F374Y/V158D/M298Q-FVII, F374Y/V158D/E296V-FVII, F374Y/V158T/S314E-FVII, F374Y/V158T/M298Q-FVII, F374Y/V158T/E296V-FVII, F374Y/E296V/S314E-FVII, F374Y/S314E/M298Q-FVII, F374Y/E296V/M298Q-FVII, F374Y/L305V/K337A/V158D-FVII, F374Y/L305V/K337A/E296V-FVII, F374Y/L305V/K337A/M298Q-FVII, F374Y/L305V/K337A/V158T-FVII, F374Y/L305V/K337A/S314E-FVII, F374Y/L305V/V158D/E296V-FVII,

F374Y/L305V/V158D/M298Q-FVII, F374Y/L305V/V158D/S314E-FVII, F374Y/L305V/E296V/M298Q-FVII, F374Y/L305V/E296V/V158T-FVII, F374Y/L305V/E296V/S314E-FVII, F374Y/L305V/M298Q/V158T-FVII, F374Y/L305V/M298Q/S314E-FVII, F374Y/L305V/V158T/S314E-FVII, F374Y/K337A/S314E/V158T-FVII, F374Y/K337A/S314E/M298Q-FVII, F374Y/K337A/S314E/E296V-FVII, F374Y/K337A/S314E/V158D-FVII, F374Y/K337A/V158T/M298Q-FVII, F374Y/K337A/V158T/E296V-FVII, F374Y/K337A/M298Q/E296V-FVII, F374Y/K337A/M298Q/V158D-FVII, F374Y/K337A/E296V/V158D-FVII, F374Y/V158D/S314E/M298Q-FVII, F374Y/V158D/S314E/E296V-FVII, F374Y/V158D/M298Q/E296V-FVII, F374Y/V158T/S314E/E296V-FVII, F374Y/V158T/S314E/M298Q-FVII, F374Y/V158T/M298Q/E296V-FVII, F374Y/E296V/S314E/M298Q-FVII, F374Y/L305V/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/K337A/S314E-FVII, F374Y/E296V/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/M298Q/K337A –FVII, F374Y/L305V/E296V/M298Q/S314E-FVII, F374Y/V158D/E296V/M298Q/K337A-FVII, F374Y/V158D/E296V/M298Q/S314E-FVII, F374Y/L305V/V158D/K337A/S314E-FVII, F374Y/V158D/M298Q/K337A/S314E-FVII, F374Y/V158D/E296V/K337A/S314E-FVII, F374Y/L305V/V158D/E296V/M298Q-FVII, F374Y/L305V/V158D/M298Q/K337A-FVII, F374Y/L305V/V158D/E296V/K337A-FVII, F374Y/L305V/V158D/M298Q/S314E-FVII, F374Y/L305V/V158D/E296V/S314E-FVII, F374Y/V158T/E296V/M298Q/K337A-FVII, F374Y/V158T/E296V/M298Q/S314E-FVII, F374Y/L305V/V158T/K337A/S314E-FVII, F374Y/V158T/M298Q/K337A/S314E-FVII, F374Y/V158T/E296V/K337A/S314E-FVII, F374Y/L305V/V158T/E296V/M298Q-FVII, F374Y/L305V/V158T/M298Q/K337A-FVII, F374Y/L305V/V158T/E296V/K337A-FVII, F374Y/L305V/V158T/M298Q/S314E-FVII, F374Y/L305V/V158T/E296V/S314E-FVII, F374Y/E296V/M298Q/K337A/V158T/S314E-FVII, F374Y/V158D/E296V/M298Q/K337A/S314E-FVII, F374Y/L305V/V158D/E296V/M298Q/S314E-FVII, F374Y/L305V/E296V/M298Q/V158T/S314E-FVII, F374Y/L305V/E296V/M298Q/K337A/V158T-FVII, F374Y/L305V/E296V/K337A/V158T/S314E-FVII, F374Y/L305V/M298Q/K337A/V158T/S314E-FVII, F374Y/L305V/V158D/E296V/M298Q/K337A-FVII, F374Y/L305V/V158D/E296V/K337A/S314E-FVII, F374Y/L305V/V158D/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/M298Q/K337A/V158T/S314E-FVII, F374Y/L305V/V158D/E296V/M298Q/K337A/S314E-FVII, S52A-Factor VII, S60A-Factor VII;

R152E-Factor VII, S344A-Factor VII, Factor VIIa lacking the Gla domain; and P11Q/K33E-FVII, T106N-FVII, K143N/N145T-FVII, V253N-FVII, R290N/A292T-FVII, G291N-FVII, R315N/V317T-FVII, K143N/N145T/R315N/V317T-FVII; and FVII having substitutions, additions or deletions in the amino acid sequence from 233Thr to 240Asn, FVII having substitutions, additions or deletions in the amino acid sequence from 304Arg to 329Cys.

5. The composition according to any one of claims 1-4, wherein said molar ratio between wild type human FVIIa and said Factor VII related polypeptide is from about 10:90 to about 90:10, such as from about 20:80 to about 80:20, such as from about 30:70 to about 70:30, such as from about 40:60 to about 60:40.

6. The composition according to any one of claims 1-4, wherein said molar ratio between wild type human FVIIa and said Factor VII related polypeptide is from about 1:99 to about 10:90, such as from about 10:90 to about 20:80, such as from about 20:80 to about 30:70, such as from about 30:70 to about 40:60.

7. The composition according to any one of claims 1-4, wherein said molar ratio between wild type human FVIIa and said Factor VII related polypeptide is from about 99:1 to about 90:10, such as from about 90:10 to about 80:20, such as from about 80:20 to about 70:30, such as from about 70:30 to about 60:40.

8. The composition according to any one of claims 1-7, wherein the ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 1.25 when tested in an In Vitro Hydrolysis Assay.

9. The composition according to claim 8, wherein said ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 2.0, such as at least about 4.0, when tested in an In Vitro Hydrolysis Assay.

10. The composition according to any one of claims 1-9, wherein the ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 1.25 when tested in an In Vitro Proteolysis Assay.

11. The composition according to claim 10, wherein said ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 2.0, such as at least about 4.0, when tested in an In Vitro Proteolysis Assay.

12. A method for the treatment of bleeding episodes in a subject or for the enhancement of the normal haemostatic system, the method comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of:

a) composition comprising wild type human FVIIa and a Factor VII related polypeptide; or

b) a first composition comprising wild type human FVIIa and a second composition comprising a Factor VII related polypeptide.

13. The method according to claim 12, wherein the molar ratio between said wild type human FVIIa and said Factor VII related polypeptide is from about 1:99 to about 99:1.

14. The method according to any one of claims 12 or 13, wherein said Factor VII related polypeptide has a proteolytic activity higher than wild type human FVIIa.

15. The method according to any one of claims 12-14, wherein said Factor VII related polypeptide is selected from the group consisting of: L305V-FVII, L305V/M306D/D309S-FVII, L305I-FVII, L305T-FVII, F374P-FVII, V158T/M298Q-FVII, V158D/E296V/M298Q-FVII, K337A-FVII, M298Q-FVII, V158D/M298Q-FVII, L305V/K337A-FVII, V158D/E296V/M298Q/L305V-FVII, V158D/E296V/M298Q/K337A-FVII, V158D/E296V/M298Q/L305V/K337A-FVII, K157A-FVII, E296V-FVII, E296V/M298Q-FVII, V158D/E296V-FVII, V158D/M298K-FVII, and S336G-FVII, L305V/K337A-FVII, L305V/V158D-FVII, L305V/E296V-FVII, L305V/M298Q-FVII, L305V/V158T-FVII, L305V/K337A/V158T-FVII, L305V/K337A/M298Q-FVII, L305V/K337A/E296V-FVII, L305V/K337A/V158D-FVII, L305V/V158D/M298Q-FVII, L305V/V158D/E296V-FVII, L305V/V158T/M298Q-FVII, L305V/V158T/E296V-FVII, L305V/E296V/M298Q-FVII, L305V/V158D/E296V/M298Q-FVII, L305V/V158T/E296V/M298Q-FVII, L305V/V158T/K337A/M298Q-FVII, L305V/V158T/E296V/K337A-FVII, L305V/V158D/K337A/M298Q-FVII, L305V/V158D/E296V/K337A–FVII, L305V/V158D/E296V/M298Q/K337A-FVII, L305V/V158T/E296V/M298Q/K337A-FVII, S314E/K316H-FVII, S314E/K316Q-FVII, S314E/L305V-FVII, S314E/K337A-FVII, S314E/V158D-FVII, S314E/E296V-FVII, S314E/M298Q-FVII, S314E/V158T-FVII, K316H/L305V-FVII, K316H/K337A-FVII, K316H/V158D-FVII, K316H/E296V-FVII,

K316H/M298Q-FVII, K316H/V158T-FVII, K316Q/L305V-FVII, K316Q/K337A-FVII, K316Q/V158D-FVII, K316Q/E296V-FVII, K316Q/M298Q-FVII, K316Q/V158T-FVII, S314E/L305V/K337A-FVII, S314E/L305V/V158D-FVII, S314E/L305V/E296V-FVII, S314E/L305V/M298Q-FVII, S314E/L305V/V158T-FVII, S314E/L305V/K337A/V158T-FVII, S314E/L305V/K337A/M298Q-FVII, S314E/L305V/K337A/E296V-FVII, S314E/L305V/K337A/V158D-FVII, S314E/L305V/V158D/M298Q-FVII, S314E/L305V/V158D/E296V-FVII, S314E/L305V/V158T/M298Q-FVII, S314E/L305V/V158T/E296V-FVII, S314E/L305V/E296V/M298Q-FVII, S314E/L305V/V158D/E296V/M298Q-FVII, S314E/L305V/V158T/E296V/M298Q-FVII, S314E/L305V/V158T/K337A/M298Q-FVII, S314E/L305V/V158T/E296V/K337A-FVII, S314E/L305V/V158D/K337A/M298Q-FVII, S314E/L305V/V158D/E296V/K337A-FVII, S314E/L305V/V158D/E296V/M298Q/K337A-FVII, S314E/L305V/V158T/E296V/M298Q/K337A-FVII, K316H/L305V/K337A-FVII, K316H/L305V/V158D-FVII, K316H/L305V/E296V-FVII, K316H/L305V/M298Q-FVII, K316H/L305V/V158T-FVII, K316H/L305V/K337A/V158T-FVII, K316H/L305V/K337A/M298Q-FVII, K316H/L305V/K337A/E296V-FVII, K316H/L305V/K337A/V158D-FVII, K316H/L305V/V158D/M298Q-FVII, K316H/L305V/V158D/E296V-FVII, K316H/L305V/V158T/M298Q-FVII, K316H/L305V/V158T/E296V-FVII, K316H/L305V/E296V/M298Q-FVII, K316H/L305V/V158D/E296V/M298Q-FVII, K316H/L305V/V158T/E296V/M298Q-FVII, K316H/L305V/V158T/K337A/M298Q-FVII, K316H/L305V/V158T/E296V/K337A-FVII, K316H/L305V/V158D/K337A/M298Q-FVII, K316H/L305V/V158D/E296V/K337A –FVII, K316H/L305V/V158D/E296V/M298Q/K337A-FVII, K316H/L305V/V158T/E296V/M298Q/K337A-FVII, K316Q/L305V/K337A-FVII, K316Q/L305V/V158D-FVII, K316Q/L305V/E296V-FVII, K316Q/L305V/M298Q-FVII, K316Q/L305V/V158T-FVII, K316Q/L305V/K337A/V158T-FVII, K316Q/L305V/K337A/M298Q-FVII, K316Q/L305V/K337A/E296V-FVII, K316Q/L305V/K337A/V158D-FVII, K316Q/L305V/V158D/M298Q-FVII, K316Q/L305V/V158D/E296V-FVII, K316Q/L305V/V158T/M298Q-FVII, K316Q/L305V/V158T/E296V-FVII, K316Q/L305V/E296V/M298Q-FVII, K316Q/L305V/V158D/E296V/M298Q-FVII, K316Q/L305V/V158T/E296V/M298Q-FVII, K316Q/L305V/V158T/K337A/M298Q-FVII, K316Q/L305V/V158T/E296V/K337A-FVII, K316Q/L305V/V158D/K337A/M298Q-FVII, K316Q/L305V/V158D/E296V/K337A –FVII, K316Q/L305V/V158D/E296V/M298Q/K337A-FVII, K316Q/L305V/V158T/E296V/M298Q/K337A-FVII, F374Y/K337A-FVII, F374Y/V158D-FVII, F374Y/E296V-FVII, F374Y/M298Q-FVII, F374Y/V158T-FVII, F374Y/S314E-FVII, F374Y/L305V-FVII, F374Y/L305V/K337A-FVII, F374Y/L305V/V158D-FVII,

F374Y/L305V/E296V-FVII, F374Y/L305V/M298Q-FVII, F374Y/L305V/V158T-FVII, F374Y/L305V/S314E-FVII, F374Y/K337A/S314E-FVII, F374Y/K337A/V158T-FVII, F374Y/K337A/M298Q-FVII, F374Y/K337A/E296V-FVII, F374Y/K337A/V158D-FVII, F374Y/V158D/S314E-FVII, F374Y/V158D/M298Q-FVII, F374Y/V158D/E296V-FVII, F374Y/V158T/S314E-FVII, F374Y/V158T/M298Q-FVII, F374Y/V158T/E296V-FVII, F374Y/E296V/S314E-FVII, F374Y/S314E/M298Q-FVII, F374Y/E296V/M298Q-FVII, F374Y/L305V/K337A/V158D-FVII, F374Y/L305V/K337A/E296V-FVII, F374Y/L305V/K337A/M298Q-FVII, F374Y/L305V/K337A/V158T-FVII, F374Y/L305V/K337A/S314E-FVII, F374Y/L305V/V158D/E296V-FVII, F374Y/L305V/V158D/M298Q-FVII, F374Y/L305V/V158D/S314E-FVII, F374Y/L305V/E296V/M298Q-FVII, F374Y/L305V/E296V/V158T-FVII, F374Y/L305V/E296V/S314E-FVII, F374Y/L305V/M298Q/V158T-FVII, F374Y/L305V/M298Q/S314E-FVII, F374Y/L305V/V158T/S314E-FVII, F374Y/K337A/S314E/V158T-FVII, F374Y/K337A/S314E/M298Q-FVII, F374Y/K337A/S314E/E296V-FVII, F374Y/K337A/S314E/V158D-FVII, F374Y/K337A/V158T/M298Q-FVII, F374Y/K337A/V158T/E296V-FVII, F374Y/K337A/M298Q/E296V-FVII, F374Y/K337A/M298Q/V158D-FVII, F374Y/K337A/E296V/V158D-FVII, F374Y/V158D/S314E/M298Q-FVII, F374Y/V158D/S314E/E296V-FVII, F374Y/V158D/M298Q/E296V-FVII, F374Y/V158T/S314E/E296V-FVII, F374Y/V158T/S314E/M298Q-FVII, F374Y/V158T/M298Q/E296V-FVII, F374Y/E296V/S314E/M298Q-FVII, F374Y/L305V/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/K337A/S314E-FVII, F374Y/E296V/M298Q/K337A/S314E-FVII, F374Y/L305V/E296V/M298Q/K337A –FVII, F374Y/L305V/E296V/M298Q/S314E-FVII, F374Y/V158D/E296V/M298Q/K337A-FVII, F374Y/V158D/E296V/M298Q/S314E-FVII, F374Y/L305V/V158D/K337A/S314E-FVII, F374Y/V158D/M298Q/K337A/S314E-FVII, F374Y/V158D/E296V/K337A/S314E-FVII, F374Y/L305V/V158D/E296V/M298Q-FVII, F374Y/L305V/V158D/M298Q/K337A-FVII, F374Y/L305V/V158D/E296V/K337A-FVII, F374Y/L305V/V158D/M298Q/S314E-FVII, F374Y/L305V/V158D/E296V/S314E-FVII, F374Y/V158T/E296V/M298Q/K337A-FVII, F374Y/V158T/E296V/M298Q/S314E-FVII, F374Y/L305V/V158T/K337A/S314E-FVII, F374Y/V158T/M298Q/K337A/S314E-FVII, F374Y/V158T/E296V/K337A/S314E-FVII, F374Y/L305V/V158T/E296V/M298Q-FVII, F374Y/L305V/V158T/M298Q/K337A-FVII, F374Y/L305V/V158T/E296V/K337A-FVII, F374Y/L305V/V158T/M298Q/S314E-FVII, F374Y/L305V/V158T/E296V/S314E-FVII, F374Y/E296V/M298Q/K337A/V158T/S314E-FVII, F374Y/V158D/E296V/M298Q/K337A/S314E-FVII, F374Y/L305V/V158D/E296V/M298Q/S314E-FVII, F374Y/L305V/E296V/M298Q/V158T/S314E-FVII,

F374Y/L305V/E296V/M298Q/K337A/V158T-FVII,
F374Y/L305V/E296V/K337A/V158T/S314E-FVII,
F374Y/L305V/M298Q/K337A/V158T/S314E-FVII,
F374Y/L305V/V158D/E296V/M298Q/K337A-FVII,
F374Y/L305V/V158D/E296V/K337A/S314E-FVII,
F374Y/L305V/V158D/M298Q/K337A/S314E-FVII,
F374Y/L305V/E296V/M298Q/K337A/V158T/S314E-FVII,
F374Y/L305V/V158D/E296V/M298Q/K337A/S314E-FVII, S52A-Factor VII, S60A-Factor VII; R152E-Factor VII, S344A-Factor VII, Factor VIIa lacking the Gla domain; and P11Q/K33E-FVII, T106N-FVII, K143N/N145T-FVII, V253N-FVII, R290N/A292T-FVII, G291N-FVII, R315N/V317T-FVII, K143N/N145T/R315N/V317T-FVII; and FVII having substitutions, additions or deletions in the amino acid sequence from 233Thr to 240Asn, FVII having substitutions, additions or deletions in the amino acid sequence from 304Arg to 329Cys.

16. The method according to any one of claims 12-15, wherein said molar ratio between wild type human FVIIa and said Factor VII related polypeptide is from about 10:90 to about 90:10, such as from about 20:80 to about 80:20, such as from about 30:70 to about 70:30, such as from about 40:60 to about 60:40.

17. The method according to any one of claims 12-15, wherein said molar ratio between wild type human FVIIa and said Factor VII related polypeptide is from about 1:99 to about 10:90, such as from about 10:90 to about 20:80, such as from about 20:80 to about 30:70, such as from about 30:70 to about 40:60.

18. The method according to any one of claims 12-15, wherein said molar ratio between wild type human FVIIa and said Factor VII related polypeptide is from about 99:1 to about 90:10, such as from about 90:10 to about 80:20, such as from about 80:20 to about 70:30, such as from about 70:30 to about 60:40.

19. The method according to any one of claims 12-18, wherein the ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 1.25 when tested in an In Vitro Hydrolysis Assay.

20. The method according to claim 19, wherein said ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human

Factor VIIa is at least about 2.0, such as at least about 4.0, when tested in an In Vitro Hydrolysis Assay.

21. The method according to any one of claims 12-20, wherein the ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 1.25 when tested in an In Vitro Proteolysis Assay.

22. The method according to claim 21, wherein said ratio between the proteolytic activity of said Factor VII related polypeptide and the proteolytic activity of the wild type human Factor VIIa is at least about 2.0, such as at least about 4.0, when tested in an In Vitro Proteolysis Assay.

23. A process for preparing a composition according to any one of claims 1-11, wherein the process comprises the step of:
mixing wild type human FVIIa with a Factor VII related polypeptide in

24. A use of a composition according to any one of claims 1-11 for the preparation of a medicament for the treatment of bleeding episodes in a subject or for the enhancement of the normal haemostatic system.

FIGURE 1 - the amino acid sequence of wild type human coagulation Factor VII. The three-letter indication "GLA" means 4-carboxyglutamic acid (γ-carboxyglutamate).

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Ala-Asn-Ala-Phe-Leu-GLA-GLA-Leu-Arg-Pro-Gly-Ser-Leu-GLA-Arg-GLA-Cys-Lys-
                  5                  10                  15

GLA-GLA-Gln-Cys-Ser-Phe-GLA-GLA-Ala-Arg-GLA-Ile-Phe-Lys-Asp-Ala-GLA-Arg-
     20                  25                  30                  35

Thr-Lys-Leu-Phe-Trp-Ile-Ser-Tyr-Ser-Asp-Gly-Asp-Gln-Cys-Ala-Ser-Ser-Pro-
             40                  45                  50

Cys-Gln-Asn-Gly-Gly-Ser-Cys-Lys-Asp-Gln-Leu-Gln-Ser-Tyr-Ile-Cys-Phe-Cys-
 55                  60                  65                  70

Leu-Pro-Ala-Phe-Glu-Gly-Arg-Asn-Cys-Glu-Thr-His-Lys-Asp-Asp-Gln-Leu-Ile-
         75                  80                  85                  90

Cys-Val-Asn-Glu-Asn-Gly-Gly-Cys-Glu-Gln-Tyr-Cys-Ser-Asp-His-Thr-Gly-Thr-
                 95                 100                 105

Lys-Arg-Ser-Cys-Arg-Cys-His-Glu-Gly-Tyr-Ser-Leu-Leu-Ala-Asp-Gly-Val-Ser-
    110                 115                 120                 125

Cys-Thr-Pro-Thr-Val-Glu-Tyr-Pro-Cys-Gly-Lys-Ile-Pro-Ile-Leu-Glu-Lys-Arg-
            130                 135                 140

Asn-Ala-Ser-Lys-Pro-Gln-Gly-Arg-Ile-Val-Gly-Gly-Lys-Val-Cys-Pro-Lys-Gly-
145                 150                 155                 160

Glu-Cys-Pro-Trp-Gln-Val-Leu-Leu-Leu-Val-Asn-Gly-Ala-Gln-Leu-Cys-Gly-Gly-
        165                 170                 175                 180

Thr-Leu-Ile-Asn-Thr-Ile-Trp-Val-Val-Ser-Ala-Ala-His-Cys-Phe-Asp-Lys-Ile-
                185                 190                 195

Lys-Asn-Trp-Arg-Asn-Leu-Ile-Ala-Val-Leu-Gly-Glu-His-Asp-Leu-Ser-Glu-His-
    200                 205                 210                 215
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Asp-Gly-Asp-Glu-Gln-Ser-Arg-Arg-Val-Ala-Gln-Val-Ile-Ile-Pro-Ser-Thr-Tyr-
            220                 225                 230

Val-Pro-Gly-Thr-Thr-Asn-His-Asp-Ile-Ala-Leu-Leu-Arg-Leu-His-Gln-Pro-Val-
235                 240                 245                 250

Val-Leu-Thr-Asp-His-Val-Val-Pro-Leu-Cys-Leu-Pro-Glu-Arg-Thr-Phe-Ser-Glu-
        255                 260                 265                 270

Arg-Thr-Leu-Ala-Phe-Val-Arg-Phe-Ser-Leu-Val-Ser-Gly-Trp-Gly-Gln-Leu-Leu-
                275                 280                 285

Asp-Arg-Gly-Ala-Thr-Ala-Leu-Glu-Leu-Met-Val-Leu-Asn-Val-Pro-Arg-Leu-Met-
    290                 295                 300                 305

Thr-Gln-Asp-Cys-Leu-Gln-Gln-Ser-Arg-Lys-Val-Gly-Asp-Ser-Pro-Asn-Ile-Thr-
            310                 315                 320

Glu-Tyr-Met-Phe-Cys-Ala-Gly-Tyr-Ser-Asp-Gly-Ser-Lys-Asp-Ser-Cys-Lys-Gly-
325                 330                 335                 340

Asp-Ser-Gly-Gly-Pro-His-Ala-Thr-His-Tyr-Arg-Gly-Thr-Trp-Tyr-Leu-Thr-Gly-
        345                 350                 355                 360

Ile-Val-Ser-Trp-Gly-Gln-Gly-Cys-Ala-Thr-Val-Gly-His-Phe-Gly-Val-Tyr-Thr-
                365                 370                 375

Arg-Val-Ser-Gln-Tyr-Ile-Glu-Trp-Leu-Gln-Lys-Leu-Met-Arg-Ser-Glu-Pro-Arg-
    380                 385                 390                 395

Pro-Gly-Val-Leu-Leu-Arg-Ala-Pro-Phe-Pro
            400                 405 406
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Fig. 1 continued

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(71) Applicant *(for all designated States except US)*: **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PERSSON, Egon** [SE/SE]; Lundavägen 60, S-232 52 Åkarp (SE). **HANSEN, Carsten, Borgund** [DK/DK]; Platanhaven 69, DK-2600 Glostrup (DK).

(74) Common Representative: **NOVO NORDISK A/S**; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FORMULATIONS COMPRISING FACTOR VIIA AND A FACTOR VII RELATED POLYPEPTIDE

(57) Abstract: The present invention relates to compositions comprising coagulation factor FVIIa, and a factor VII related polypeptide.

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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/66031 A (MATTHIESSEN PETER ; BAXTER AG (AT); TURECEK PETER (AT); SCHWARZ HANS P) 23 December 1999 (1999-12-23) page 2, paragraph 5 - page 3, paragraph 3 -/--	1,2,6, 12,13, 17,24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Escolar Blasco, P

INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NELSESTUEN G L ET AL: "EleVated Function of Blood Clotting Factor VIIa Mutants That Have Enhanced Affinity for Membranes" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 276, no. 43, 26 October 2001 (2001-10-26), pages 39825-39831, XP002254308 ISSN: 0021-9258 page 39826, left-hand column, last paragraph - right-hand column, last paragraph; figure 3 page 39831, left-hand column, paragraph 2	1,2
X	WO 03/027147 A (NOVO NORDISK AS) 3 April 2003 (2003-04-03) page 4, line 33 - page 27, line 33; examples 5,6	1-24
A	SHAH A ET AL: "Manipulation of the membrane binding site of vitamin K-dependent proteins: enhanced biological function of human factor VII" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 95, no. 8, 14 April 1998 (1998-04-14), pages 4229-4234, XP002092845 ISSN: 0027-8424 Discussion	1-24
A	PERSSON EGON ET AL: "Assignment of molecular properties of a superactive coagulation factor VIIa variant to individual amino acid changes." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 269, no. 23, December 2002 (2002-12), pages 5950-5955, XP002309086 ISSN: 0014-2956 abstract page 5953, left-hand column, lines 15-19	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2004/000413

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [X] in computer readable form

   c. time of filing/furnishing

   [ ] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [X] furnished subsequently to this Authority for the purpose of search

2. [X] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2004/000413

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 12-22: Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK2004/000413

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